The response of human muscle triceps brachii (TB) to training, which consisted of skiing with sledges 500 miles over a period of 36 days, has been investigated by means of histochemical fiber typing (myofibrillar ATPase, $n = 7$). Muscle biopsies were taken in the right TB during pretraining, and in the right as well as in the left TB after training. The percentage of type 2 fibers and intermediate fibers (type 2C and 1B) in the right TB decreased and increased, respectively, by 13%. The fiber type distribution in the left TB after training was very similar to that in the right TB after training. This concordance indicates that the change in fiber type distribution was a result of the training per se, and not related to any eventual effect of the biopsy-sampling.

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INCREASES IN MYOFIBRILLAR ATPase INTERMEDIATE HUMAN SKELETAL MUSCLE FIBERS IN RESPONSE TO ENDURANCE TRAINING

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In a recent report in this journal,[24] the authors described an increased percentage of myofibrillar ATPase intermediate fibers (type 2C and 1B) in the human triceps brachii muscle (TB) in response to prolonged endurance training. Based on these results and on information about the nature of myofibrillar ATPase intermediate fibers,[2,14–16,20,22] the increased fraction of intermediate fibers was interpreted as a sign of ongoing fiber type transformation.

The authors could not, however, entirely rule out that the increase of intermediate fibers was due to repeated biopsy-sampling which caused nerve damage, followed by reinnervation,[15,19] or fiber damage.[13,23] Although this seemed unlikely, since there were no signs of muscle denervation (atrophy), reinnervation (type grouping), or muscle fiber regeneration (myotubes),[12,18,23] it was considered necessary to examine this possibility. A control study was, therefore, undertaken. The fiber type distribution was studied in the right TB before prolonged endurance training, and in the right and left TB after training.

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MATERIALS AND METHODS

Subjects. Four healthy, moderately trained women and three men participated in the study. Average age, height, and weight for the women was 25 years (range 20–29), 1.62 m (1.60–1.70), and 58 kg (50–64), respectively, and for the men 27 years (24–29), 1.81 m (1.73–1.98), and 80 kg (68–98), respectively. They were informed about the procedure and the risks involved in the experiments before they volunteered to participate. The study was approved by the Committee of Ethics at the Karolinska Institutet.

Training. The training consisted of approximately 500 miles (800 km) of skiing in mountain terrain. Teams of two skiers pulled a load (80 kg) on one sledge. The distance was covered in 36 days of skiing with 5 days of rest evenly interspersed.

Muscle Sampling and Staining. Muscle biopsies were obtained from the middle-lower portion of the medial head of TB using the needle biopsy technique.[1] Biopsies were taken from the right TB before and after training, as well as from the left TB after training. The posttraining biopsies were taken 5 days after termination of the training. Serial transverse sections of the muscle samples were stained for myofibrillar ATPase activity.[10,21] The fibers were classified into fiber types 1 and 2[8] and

into the subgroups 2A and 2B,[4] type 2C,[5,6] and 1B.[7] Type 2C and 1B fibers will be referred to as intermediate fibers. On the average, 420 (range 130–920) fibers were counted from each muscle before and after training. The handling of the biopsies, the staining procedure, as well as the fiber type classification has been described in detail elsewhere.[24]

Statistics. Results are presented as means and standard error of means or ranges. A two-way analysis of variance (ANOVA) of no difference in means was applied to the data on fiber type distribution. Tukey's method of multiple comparisons[17] was applied when the ANOVA indicated an overall significance. The magnitude of the variance differed for the percentage of intermediate fibers before and after training, hence the ANOVA could not be applied on this portion of fibers. Instead, Wilcoxon's signed rank sum test was used for comparisons. In that test, because the different portions of intermediate fibers were used in two comparisons, a higher significance level ($P < 0.025$) was chosen. In other tests, a probability level of less than 0.05 was considered significant.

RESULTS

The muscle fiber type distribution before and after training is listed in Table 1. No change occurred in training with respect to the type 1 fibers, while the percentage of type 2 fibers (2A + 2B) decreased from 69% before training to 56% after training in the right TB. The same percentage (56%) was seen in the left TB after training. It differed significantly from the percentage of type 2 fibers seen in the right TB at pretraining. The decrease in type 2 fibers was associated with an increase in intermediate fibers (IM) from 2% to 15%. The percentage of IM fibers in the left TB after training was similar to that in the right TB (14%), but did not differ significantly from the percentage of IM fibers seen in the right TB at pretraining. Individual changes in percentages of IM fibers are depicted in Fig. 1. The IM fibers were almost exclusively of the 2C type,[5,6] i.e., darkly stained for myofibrillar ATPase after alkaline preincubation, but intermediately stained after acid preincubation (Fig. 2). No morphological abnormalities were noted upon light microscopical inspection of the myofibrillar ATPase stainings, nor was there any obvious fiber type grouping evident.

Table 1. Muscle fiber type distribution (%) in TB before and after training (means ± SEM).

Fiber types	Before training	After training	
	right	right	left
1	29 ± 3	28 ± 2	30 ± 3
Intermediate	2 ± 1	*15 ± 4	14 ± 4
2A	48 ± 4	42 ± 5	37 ± 4
2B	21 ± 5	14 ± 3	19 ± 5
(2A + 2B)	69 ± 3	*56 ± 5	*56 ± 5

*Denotes significant differences ($P < 0.05$) versus pretraining values.

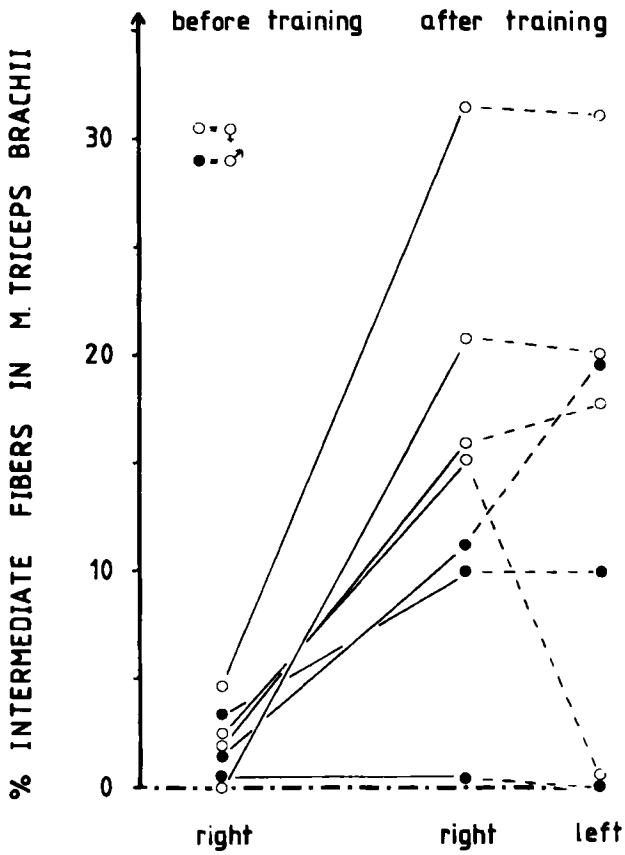

Figure 1. Individual percentages of intermediate fibers in the right and left TB before and after training.

DISCUSSION

The present results provide two forms of evidence that the difference in fiber type distribution in the right TB before and after training is a result of the training program per se, and not related to nerve or fiber damage due to the biopsy sampling. The first form of evidence is that in five out of the six subjects in whom substantial increases of intermediate fibers are seen in the right TB, increases are also seen when comparing the left TB after training with the right TB at pretraining. The second form of evidence is that the posttraining fiber type distributions are similar in the right and left TB. A prerequisite for the preceding comparisons

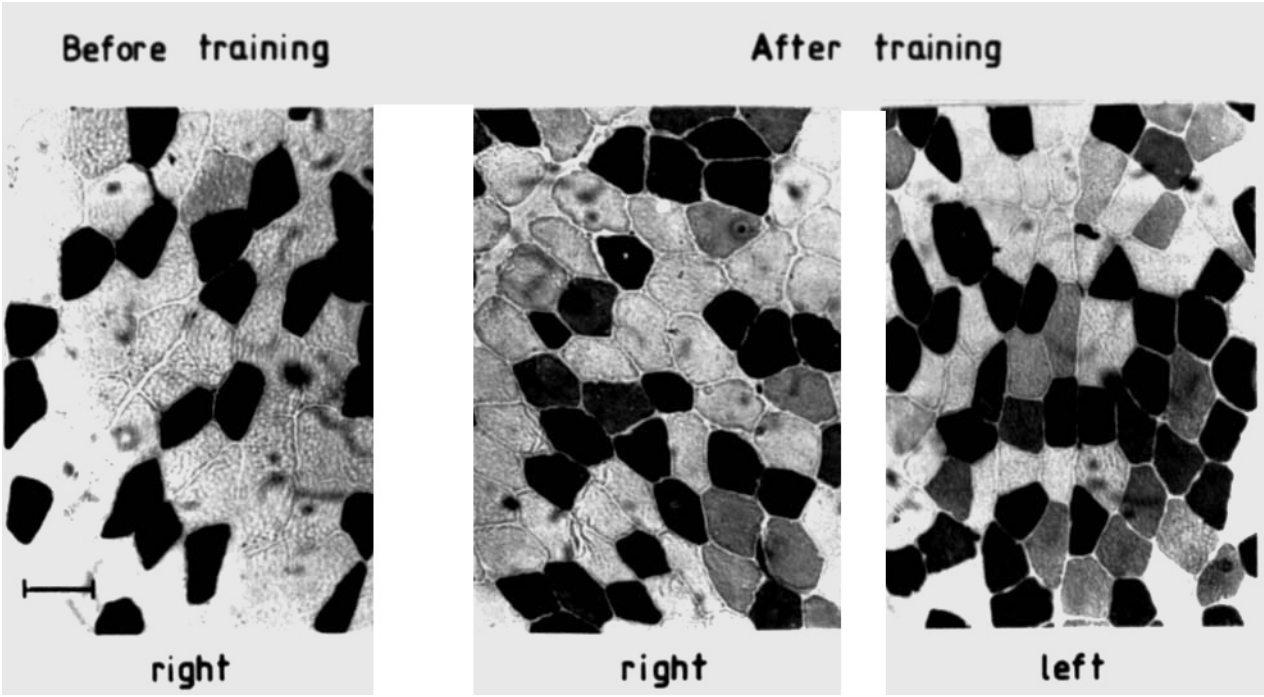

Figure 2. Staining for myofibrillar ATPase after preincubation at pH 4.3. The pictures were taken of samples from TB of a female subject in whom the percentage of intermediate fibers increased markedly during training. The intermdiate fibers were, with very few exceptions, of the 2C type (for references see text). After preincubation at pH 4.3, the type 2C fibers stain intermediately and are distinguishable from the other fiber types. (Type 1 fibers stain dark, type 2 fibers are unstained.) The length of the calibration bar corresponds to 0.1 mm.

lies in the assumption that no difference existed between the right and left TB at pretraining. Although a difference in the distribution of type 1 and type 2 fibers has been noted between some dominant and nondominant hand and forearm muscles,[9,11] a right-left symmetry has been shown in the upper arm and shoulder muscles, including TB,[11] as well as in leg muscles.[3] Furthermore, the authors have previously determined the fiber type distribution in the left TB of six untrained individuals.[24] No intermediate fibers were observed. In the present study, the same frequency of type 1 fibers were seen in the right TB before and after training as in the left TB after training. Thus, the prerequisite for the present comparison seems to be fulfilled.

If the biopsy-taking procedure has influenced or caused the increase in intermediate fibers in the right TB, a greater percentage of intermediate fibers would be expected in the right compared to the left TB after training. The risk that such a difference actually exists is not negligible. However, if the biopsy procedure has caused the increase in intermediate fibers, a decrease in both type 1 and type 2 fibers would be expected. As in the former study, which showed an increase in the intermediate fiber type after endurance training,[24] the increase in intermediate fibers was balanced by a decrease in type 2 (A + B) fibers. Thus, with the preceding presented forms of evidence considered together, it seems hardly likely that the increased frequency of intermediate fibers is provoked or affected by the biopsy-taking. It is, therefore, concluded that prolonged endurance training may induce transformation of type 2 fibers into intermediate fibers.

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